CHEST

Original Research

SMOKING CESSATION

A Pilot Study To Examine the Effects of Smoking Cessation on Serum Markers of Inflammation in Women at Risk for Cardiovascular Disease

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Background: **The links among smoking, inflammation, and cardiovascular disease (CVD) are well established. Several studies have demonstrated that quitting smoking reverses the risk of coronary heart disease within 5 to 10 years. However, the immediate effects of quitting smoking on inflammatory biomarkers associated with CVD risk have not been well described.**

Methods: **In this pilot study, we examined a panel of circulating inflammatory biomarkers associated with CVD in "at-risk" women during the smoking cessation program. Forty-six women enrolled in a smoking cessation program consented to attend four study visits over 6 to 7 weeks. Health/medical information and blood were collected at each visit. Circulating levels of C-reactive protein (CRP), tumor necrosis factor (TNF), interleukin (IL)-6, soluble TNF receptor (sTNFR)-I, sTNFR-II, and soluble vascular cell adhesion molecule (sVCAM)-1 were measured, and changes between baseline levels (visit 1, while smoking) and visits 2 through 4 were determined.**

Results: **Significant reductions in circulating levels of TNF, sTNFR-I, sTNFR-II, and sVCAM-1 were observed among participants over the course of the smoking cessation program. Serum levels of both IL-6 and CRP declined during the smoking cessation program; the changes were not statistically significant, however.**

Conclusions: **These findings suggest there are rapid consequences of smoking cessation on inflammatory biomarkers in women at risk for CVD. Additional, larger studies including diverse smokers desiring to quit are required to confirm changes in "measurable milestones" that could serve as motivating factors to assist smokers to quit.** ***(CHEST 2009; 136:212–219)***

Abbreviations: BMI = body mass index; CO = carbon monoxide; CRP = C-reactive protein; CVD = cardiovascular disease; IL = interleukin; IQR = interquartile range; NRT = nicotine replacement therapy; RMANCOVA = repeated measures analysis of covariance; SF-36 = Medical Outcomes Study 36-item short form; sTNFR = soluble tumor necrosis factor receptor; sVCAM = soluble vascular cell adhesion molecule; TNF = tumor necrosis factor

Smoking promotes enhanced production of proinflammatory molecules by numerous cell types[1–6] and contributes to systemic inflammation with elevated levels of inflammatory biomarkers.[7,8] Numerous studies[8–16] have identified serum biomarkers (*eg*, C-reactive protein [CRP], interleukin [IL]-6, tumor necrosis factor [TNF], soluble TNF receptors [sTNFRs] I and II) that predict the risk of COPD[9–11] and cardiovascular disease (CVD).[8,12–16] By contrast, smoking cessation is associated with improved COPD[17] and CVD risk/mortality.[18,19] However, it is not clear whether the benefits of quitting smoking are achieved immediately or require years.[20,21] The purpose of this pilot study was to investigate the effect of quitting smoking on serum inflammatory biomarkers associated with CVD in women at risk for CVD during smoking cessation.

Materials and Methods

Subjects

The institutional review board approved this study, and all subjects gave written informed consent prior to study procedures. Subjects were recruited from the North Shore-LIJ Health System "Quit for Keeps" smoking cessation program (a behavioral

Table 1—*Description of Study Visits for Subjects Attending Smoking Cessation Program*

Variables	Study Visit			
	1	2	3	4
Subjects, No.	46	42	39	36
Smoking	+	0	0	0
Cessation aids	0	+	+	+
Height	+	0	0	0
Weight	+	+	+	+
BP	+	+	+	+
Exhaled CO	+	+	+	+
SF-36	+	0	+	0
Questionnaires	+	0	0	0
Review smoking history	+	+	+	+
Review medication history	+	+	+	+
Blood collection	+	+	+	+

Study subjects attended four study visits over a 6- to 7-week smoking cessation program. + = yes or assessed; 0 = no or not assessed.

support program) between July 2005 and June 2007. Women smokers were enrolled in the study if they were at risk for CVD, which was defined as having one or more of the following: abdominal obesity; elevated cholesterol levels; high BP and/or history of heart disease; had smoked approximately 1 pack of cigarettes per day for the past year (with exhaled carbon monoxide [CO] concentration of > 15 ppm); were relatively healthy; and wanted to quit smoking. Subjects were excluded if they were receiving antiinflammatory agents (including oral corticosteroids), were currently pregnant or trying to conceive, were drinking beyond moderation, or were using other tobacco products.

Study Design

Subjects consented to attend multiple visits that overlapped with the Quit for Keeps program (6 to 8 weeks) [Table 1]. At visit 1, subjects (n = 46) were smoking and chose smoking cessation aids (*eg*, nicotine replacement therapy [NRT] and/or bupropion [Wellbutrin; GlaxoSmithKline; Research Triangle Park, NC]), which were provided free of charge), with the help of trained nurses. Subjects completed questionnaires and the Medical Outcomes Study 36-item short form (SF-36) [physical and mental health assessment] at visits 1 and 3. Height, weight, body mass index (BMI), BP, and smoking status (assessed using the measurement of expired CO and serum cotinine levels, and self-report) were recorded at each visit. Visit 2 was within 24 to 72 h of their last cigarette (and 1 week after visit 1), visit 3 was 1 to 2 weeks after visit 2, and visit 4 was 3 to 5 weeks later. Peripheral blood was collected at each visit; serum was isolated, aliquoted, and stored at −80°C until analyses of biomarkers and cotinine were performed.

Analysis of Inflammatory Biomarkers

Serum CRP levels were determined using ultrasensitive nephelometry. Serum IL-6 and TNF levels were determined using high-sensitivity enzyme-linked immunosorbent assays; serum sTNFR-I, sTNFR-II, and soluble vascular cell adhesion molecule (sVCAM)-1 levels were determined by enzyme-linked immunosorbent assays (R&D Systems; Minneapolis, MN). All serum samples were analyzed in duplicate, and samples for each subject (visits 1 through 4) were on the same plate with standards. The coefficients of variation for interassay and intraassay variability for the inflammatory markers were between 1% and 5%, and 2% and 8%, respectively.

Statistical Analysis

Statistics were analyzed using a statistical software package (SAS/PC, version 9.1; SAS Institute; Cary, NC).

Inflammatory Markers

Repeated measures analysis of covariance (RMANCOVA), where time (visits 2, 3, and 4) was the within-subjects effect (and there was no between-subjects effect), was used to examine changes from baseline (at visit 1, while smoking) and visits 2 through 4 for all subjects who completed four visits. For each biomarker, the ratio of levels of that marker to baseline levels was calculated, and then the log transformation was used to meet the assumptions of the RMANCOVA model. CO levels at each visit and BMI (at baseline) were included as covariates to control for potential effects on biomarkers. Exhaled CO was used as a surrogate marker for smoking status because it significantly associated with self-reported smoking (and NRT interfered with serum cotinine assays). To measure the degree of correlation between the self-reported number of cigarettes smoked per day and CO levels, the Spearman correlation coefficient was calculated for visits 2 through 4 (range, 0.47 to 0.62). At visit 4, all subjects who reported not smoking exhaled ≤ 8 ppm CO. On finding a significant effect of time on biomarker status, pairwise comparisons of visits 2 through 4 were carried out (RMANCOVA). To determine whether the change from baseline was significant, the level of each marker at each visit was compared to baseline levels by testing whether the ratio of visit x-to-visit 1 differed from 1 using the *t* test within the RMANCOVA model. For both comparisons, a Bonferroni adjustment was used, such that these comparisons were considered significant with $p < 0.0167$. Additional models were examined (*eg*, using serum cotinine levels as a surrogate for smoking instead of expired CO levels), and the results did not differ qualitatively from the reported results.

Feelings of Wellness (SF-36)

RMANCOVA, in which the visit was the "within-subjects effect" (and there was no "between-subjects effect"), was used to

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Table 2—*Baseline Characteristics of the Study Population*

Characteristics (n = 36)	Mean (SD)	Median (IQR)	%
Age, yr	53.9 (9.1)	54.4 (14.3)	
BMI, kg/m^2	30.0 (5.8)	29.4 (7.2)	
Systolic BP*	130.2 (13.84)	130.0 (19.0)	
Diastolic BP*	77.5 (9.1)	80.0 (12.0)	
Smoking status			
Cigarettes smoked/d	22.8 (5.7)	20.0 (0.0)	
Pack-yr	39.0 (13.53)	38.0 (14.0)	
Total SF-36 score	74.4 (14.0)	76.4 (20.4)	
Physical health score	72.0 (15.3)	75.4 (25.8)	
Mental health score	72.5 (13.9)	73.8 (22.6)	
Ethnicity			
White			58
African-American			11
Hispanic			3
Unknown			28
CVD risk factors			
Elevated cholesterol			61.1
Hypertension*			36.1
Abdominal obesity			19.4
Coronary artery disease			13.9

*Subjects with high BP were receiving antihypertensive medications.

examine the pattern of SF-36 scores during smoking cessation. In addition, a RMANCOVA model was used to test for associations between each marker and SF-36 scores (total, physical, and mental health scores). Baseline SF-36 scores (mental, physical, and total) for subjects who completed fewer than four visits were compared with those who completed four visits using the exact Mann-Whitney test.

Results

Characteristics of the Study Population

Of the 46 female participants (mean [± SD] age, 54.1 ± 9.1 years) who smoked, 36 (78.3%) completed four study visits and were included in the analyses. All subjects quit smoking at least 72 h prior to visit 2. Baseline characteristics are shown in Table 2. The baseline mean (± SD) SF-36 scores for physical and mental health were 72.0 ± 15.3 and 72.5 ± 13.9, respectively, and these values did not significantly change over the course of the study. Many subjects had multiple risk factors for CVD (in addition to smoking) [Table 2]. NRT (nicotine patch [14 or 21 mg], nicotine gum, nasal spray, inhalers, and lozenges [Cardinal Health; Dublin, OH]) and bupropion or NRT alone were used as smoking cessation aids (Fig 1).

Approximately 85% of the subjects who completed four study visits quit smoking. Those subjects who relapsed (six subjects; 15%) and continued in the study reported smoking fewer cigarettes; when these six subjects were excluded from the analyses, the

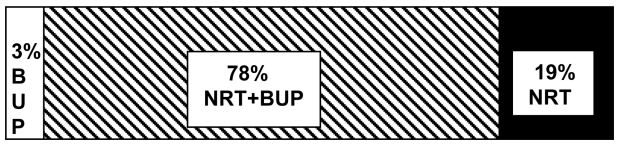

Figure 1. Smoking cessation aids used by study subjects. Most study subjects used NRT (97%), the majority of individuals used NRT + bupropion (BUP), and 19% used NRT alone.

results were similar to those observed for all subjects who had attended four study visits. Several subjects (n = 10) did not attend visit 4 due to relapse. Two differences were noted between subjects who did not complete four visits and those who did. Women who completed three visits or fewer had the following: (1) significantly lower median quality-of-life (SF-36) scores (mental score, 61.8 [interquartile range (IQR), 31.4]; total score, 62.6 [IQR, 27.9]) than women who completed four visits (mental score, 73.8 [IQR, 22.6]; total score, 76.4 [IQR, 20.4]; $p < 0.01$ and $p < 0.02$, respectively); and (2) were less likely to use smoking cessation aids.

Serum Biomarkers Associated With CVD Decline During Smoking Cessation

Based on the evidence that circulating TNF is associated with cardiovascular mortality/risk,[7,22] we examined serum TNF levels during smoking cessation. The mean baseline serum TNF concentration (1.213 ± 0.703 pg/mL) in our subjects was higher than TNF concentrations measured at later visits (Table 3). Overall, there was a decline in serum TNF concentrations over the course of the smoking cessation program among women who were at risk for CVD with a significant difference between circulating TNF concentrations observed at visits 2 and 3 ($p < 0.0115$) [Table 3].

Circulating sTNFR-I and sTNFR-II levels are significantly predictive indicators of coronary heart disease in women.[12] Steady declines in serum sTNFR-I and sTNFR-II levels were observed after quitting smoking ($p < 0.0121$ and $p < 0.0023$, respectively) [Table 3]. The serum sTNFR-I level was lower at visit 4 than at visits 1, 2, and 3, and the average sTNFR-II level at visit 4 was significantly lower than at visits 1 and 3 (Table 3). The mean baseline serum sTNFR-I concentration in our subjects (2,667.9 ± 1066.4 pg/mL) was higher than the levels described for healthy women (1,267 ± 354 pg/mL), whereas the average baseline sTNFR-II level among our subjects (2,395.1 ± 993.1 pg/mL) was comparable to that of healthy control subjects.[12]

Endothelial cell activation is proposed to play a role in the development of coronary artery disease

Table 3—*Inflammatory Markers Before and After Smoking Cessation*

Markers	Baseline Value	Visit	Mean Change From Baseline, % (95% CI)	p Value
TNF	1.213 ± 0.703 pg/mL	V1	0	
		V2	− 14.5 (− 49.8 to 45.5)	
		V3	− 27.7 (− 57.6 to 23.0)	< 0.0115 (vs V2)
		V4	− 20.2 (− 53.1 to 35.9)	
sTNFR-I	2,667.9 ± 1,066.4 pg/mL	V1	0	< 0.0001 (vs V4)
		V2	− 3.0 (− 7.9 to 2.2)	< 0.01 (vs V4)
		V3	− 3.9 (− 8.7 to 1.2)	< 0.0068 (vs V4)
		V4	− 11.6 (− 16.1 to − 7.0)	
sTNFR-II	2,395.1 ± 993.1 pg/mL	V1	0	< 0.0048 (vs V4)
		V2	− 10.4 (− 18.9 to − 1.0)	NS (vs V4)
		V3	− 3.2 (− 12.3 to 6.9)	< 0.0021 (vs V4)
		V4	− 13.7 (− 21.8 to − 4.7)	
sVCAM-1	310.1 ± 119.3 ng/mL	V1	0	< 0.0001 (vs V4)
		V2	− 0.6 (− 8.8 to 8.3)	< 0.0001 (vs V4)
		V3	− 4.1 (− 11.9 to 4.4)	< 0.0001 (vs V4)
		V4	− 19.5 (− 26.0 to − 12.3)	
IL-6*	3.314 ± 2.873 pg/mL	V1	0	NS
		V2	15.0 (− 10.8 to 48.4)	
		V3	0.7 (− 21.8 to 29.7)	
		V4	− 9.6 (− 29.8 to 16.4)	
CRP*	0.462 ± 0.519 mg/dL	V1	0	NS
		V2	− 46.5 (− 75.7 to 18.0)	
		V3	− 54.4 (− 79.1 to − 0.3)	
		V4	− 19.1 (− 63.0 to 76.8)	

Values are given as the mean ± SD, unless otherwise indicated. Baseline inflammatory marker levels and the mean percentage changes in inflammatory markers between each study visit 2 through 4 vs baseline (visit 1) are shown with the level of significance. CI = confidence interval; NS = not significant; V = visit.

*Pairwise comparisons of visits 2 through 4 were not carried out due to nonsignificant findings.

and smoking-induced endothelial cell dysfunction.[14,23] We found a significant decrease in levels of serum sVCAM-1, a marker of endothelial cell activation, after quitting smoking ($p < 0.0001$). Serum sVCAM-1 levels were significantly lower at visit 4 than at visits 1, 2, and 3 (Table 3).

Although serum levels of both IL-6 and CRP are considered to be predictors of cardiovascular events in some studies,[12] others have reported either weak or no association between IL-6 and CRP values and CVD[15,16] or CVD severity.[24] In this study, we found an insignificant reduction in serum IL-6 and CRP levels during smoking cessation.

We observed variable responses among subjects. Some subjects showed steady declines in serum biomarker levels over the course of the smoking cessation program (Fig 2). Others exhibited either no pattern or no change in circulating biomarker levels (Fig 2).

Discussion

Smoking has deadly consequences. The long-term health benefits of smoking cessation for every age group are indisputable. The ultimate goal of smoking cessation programs is to assist smokers in quitting smoking and remaining smoke free. A very recent study[25] reported that informing smokers of the "age and health" of their lungs (based on spirometric assessment) significantly improved quit rates. We propose a similar program focused on improving cardiovascular health for smokers. To develop a smoking cessation program centered on a "healthy heart," it is necessary to identify measurable early biomarkers (in men and women) associated with cardiovascular risk that are sensitive to change with smoking cessation. Once established, these biomarkers can be assessed before and during smoking cessation. Quantifiable information reflecting cardiovascular health may act as positive reinforcement for those trying to quit and remain smoke free. Similarly, biomarkers (indicative of lung dysfunction) could be determined for smokers with compromised lung function to establish smoking cessation programs focused on "healthy lungs."

Several studies[13,26,27] have reported elevated levels of inflammatory biomarkers among smokers vs those for nonsmokers. Others[21,28] have described a decline in inflammatory biomarker levels at some point after quitting smoking. Twenty years is required to reverse CRP, fibrinogen, and fibrin levels of smokers to levels found in nonsmokers.[21] Similarly, 10 years is

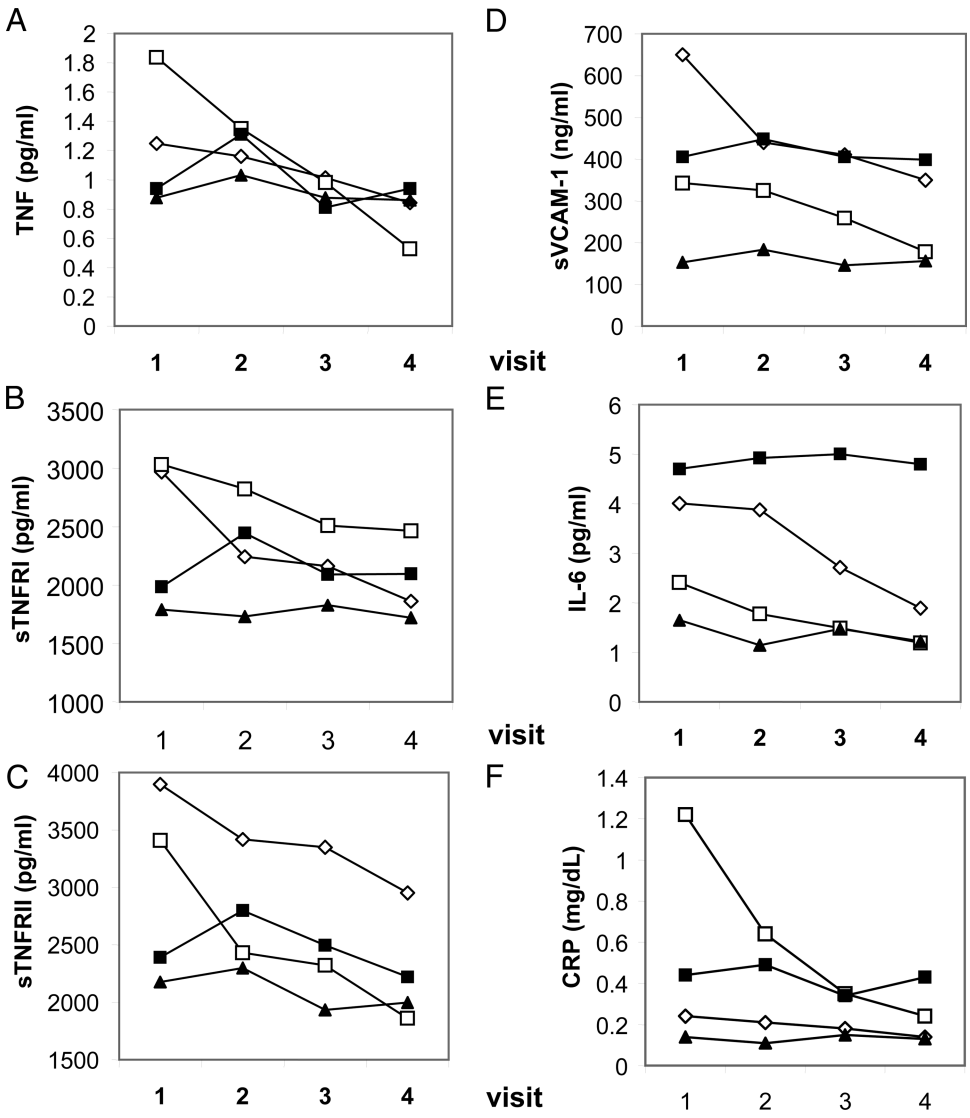

FIGURE 2. Individual subject patterns of inflammatory mediators during the smoking cessation program. Individual subject patterns of serum inflammatory mediators (associated with increased risk of CVD) during the smoking cessation process (visits 1 through 4) are shown for TNF (*A*), sTNFR-I (*B*), sTNFR-II (*C*), sVCAM-1 (*D*), IL-6 (*E*), and CRP (*F*). Open symbols denote mediator values for individual subjects (taking NRT + bupropion) whose inflammatory mediators declined over the smoking cessation program; closed symbols denote values for individual subjects where no changes in inflammatory mediator levels were observed.

required to observe a 50% reduction in inflammatory/hemostatic markers after quitting smoking.[29] These improvements in biomarker scores translate to a decline in cardiovascular risks ranging between 5 and 10 years for reducing the risk of myocardial infarctions to that of nonsmokers.[30,31] Likewise, a recent report[19] showed that women gained 61% of the full benefit of quitting smoking with regard to coronary disease mortality within 5 years of quitting.

To our knowledge, this is the first study to examine serum biomarkers associated with inflammation in women smokers at risk for CVD during smoking cessation. Based on our experience with smokers who complained of physical feelings that mimicked flu-like symptoms immediately after quitting, we hypothesized that smoking cessation might produce an initial spike in levels of inflammatory markers (immediately after quitting) reminiscent of "serum-sickness" with elevated circulating cytokine levels found after experimental administration of endotoxin. This undesirable physical feeling would be an obstacle to quitting. In several subjects, we observed a slight (insignificant) increase in biomarker levels at visit 2 (compared to baseline). These data suggest the following: (1) there is no sharp inflammatory "peak" after quitting smoking; (2) the immediate rise

was not found because blood was not sampled within a narrow time frame of quitting; (3) NRT (used by 97%) blunted inflammatory responses; and/or (4) variable responses to smoking cessation occur and a much larger population is required to examine these variable responses.

We further hypothesized that an early spike in circulating levels of inflammatory mediators after quitting smoking would be followed by steady declines. This proposed decrease is based on previous reports showing that smoking enhances systemic inflammation,[7,8] quitting is associated with declines in inflammatory biomarker levels,[21,28] and that nicotine (used to relieve cravings) exerts antiinflammatory effects in patients with ulcerative colitis[32–34] and in experimental models of systemic inflammation (*eg*, endotoxemia,[35] sepsis,[36] and ischemia[37,38]).

Based on data obtained from 36 subjects at risk for CVD who completed four visits, we found significant changes in serum levels of TNF, sTNFR-I, sTNFR-II, and sVCAM-1 from baseline (Table 3). Although no significant changes in serum levels of CRP and IL-6 levels were observed (Table 3), serum CRP levels declined slightly during smoking cessation. Our observations are consistent with a report showing that a significant reduction in serum CRP levels among heavy smokers following smoking cessation requires 5 to 9 years; 20 years are needed to reverse serum CRP levels to those found among never-smokers.[21] Together, these findings suggest that CRP, a stable downstream inflammatory marker, may not be useful as an early milestone for smokers. CRP synthesis is determined by levels of IL-6 and, to a lesser extent, of TNF,[39] which are elevated among smokers[40,41] and have potential prognostic value in predicting cardiovascular health.[42] We observed a significant decrease in serum TNF levels after quitting smoking but no significant decline in IL-6 levels (Table 3).

The physiologic response of circulating TNF is mediated through TNFR-I and TNFR-II, which are shed from circulating leukocytes during inflammation (sTNFR-I and sTNFR-II). sTNFRs, significant markers of coronary heart disease for women,[12] act as slow-release reservoirs of bioactive TNF and extend its half-life.[43] Thus, circulating sTNFR levels reflect a systemic pan-inflammatory state better than individual short-lived cytokine levels and should be better predictors of inflammation than TNF.[43] Interestingly, a recent study[44] implicates sTNFR-I and sTNFR-II in the pathogenesis of COPD. Together with our observations, these findings suggest that quitting smoking could improve inflammatory responses systemically and within the lung.

Our quit rate was 65% and 83.3%, respectively, among all subjects and subjects who completed visit 4. The major differences between subjects who completed four visits vs those who completed three or fewer visits were their SF-36 scores and use of smoking cessation aids. Our results suggest that higher mental and physical SF-36 scores are associated with a greater likelihood of attending classes, increased use of smoking cessation aids, and quitting smoking. Thus, smokers with low SF-36 scores may need additional support to quit or might want to postpone quitting until their SF-36 scores are elevated.

This study has several limitations. Although it was designed and funded to be a pilot study enrolling women only, the most critical limitation was the small sample size. This, in part, was related to US Food and Drug Administration approval of varenicline (Chantix; Pfizer; New York, NY) in May 2006. Although we had a smaller than anticipated enrollment, most of our subjects (97%) used NRT, and 78% of the subjects completed four visits. SF-36 scores did not change significantly with biomarker levels during the smoking cessation program. The SF-36 form is recommended for the assessment of general health,[45] but a major disadvantage is that it may not be sensitive to short-term changes in feelings of wellness experienced by our subjects who were relatively healthy. In addition, the smokers might have reduced smoking prior to visit 1, which might have reduced baseline values. Assessment of smoking status based on exhaled CO concentrations and self-report is another limitation. The serum cotinine level was measured throughout the study, but values were confounded by NRT (and for ethical reasons subjects were encouraged to continue their NRT). It is important to note that variable responses in inflammatory markers were observed. Variability might be associated with ethnicity, health status, smoking cessation aids (although 97% of our subjects used NRT), genetics, and age. Areas of future investigation include determining the sources of variability in inflammatory responses during smoking cessation and whether this variability influences successful long-term smoking cessation.

CVD is the most common cause of preventable death among adult Americans. Smoking as few as one to four cigarettes to one pack per day significantly increases the risk of fatal coronary heart disease by more than twofold and fivefold, respectively.[46] Each year more women die from CVD than men.[47] Although numerous CVD risk factors have been identified, poor implementation of programs to reduce these risk factors in women has been widely documented.[48,49] Because of these factors and the reported gender differences in smoking cessation rates, with women having less success than men,[50,51] we included women who were at risk for CVD in our study.[51] Thus, until a similar study can be repeated

with a much larger, more diverse population, our results can be only generalized to women at risk for CVD.

The development of successful smoking cessation programs for men and women is critical to reduce the number of deaths associated with smoking. We propose the identification of a panel of inflammatory biomarkers that could be used as measurable milestones for persons quitting smoking in a smoking cessation program focused on improving cardiovascular health for smokers who are at risk. This quantifiable information reflecting cardiovascular health may serve as positive reinforcement for those trying to quit smoking and remain smoke free. Likewise, a panel of markers could be established for smokers with abnormal lung function.

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